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16. ABSTRACT

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Hazard Assessment

A LITTORAL ENCLOSURE FOR REPLICATED FIELD EXPERIMENTS

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Abstract—A design for 5 × 10 m littoral enclosures that extend 10 m from the shoreline into the zone of submergent vegetation and incorporate undisturbed natural sediments for the bottom is presented and construction techniques are described. This type of enclosure was used to study the responses of caged and free-living pond biota and the physical and chemical environment to a single application of Dursban® (chlorpyrifos) during the summer of 1986 as part of the development of a field testing protocol for pesticides needed by the U.S. Environmental Protection Agency Office of Pesticide Programs. Chlorpyrifos was added to 12 littoral enclosures built within a mesotrophic, 2-ha pond near Duluth, Minnesota, at nominal concentrations of 0.0, 0.5, 5.0 or 20.0 μg/L. The enclosures proved to be both economical and durable and were useful for detecting direct and indirect (ecological) effects of the pesticide. Coefficients of variation (C.V.) associated with the chemical and biological response variables typically ranged from 10 to 40%. The ability to simultaneously monitor many response variables in replicate enclosures at a relatively low cost suggests that the littoral enclosure design should be useful for studying the effects of pesticide or other pollutant additions to natural aquatic systems.

Keywords—Littoral enclosures Replication Field experiments Pesticides Coefficient of variation

INTRODUCTION

During the past 30 years, a wide variety of enclosures have been used to study physical, chemical and/or biological responses to chemicals or other experimental treatments in natural aquatic systems [1–8]. These enclosures have varied in shape, size, wall material, bottom type and location within the water body. They usually have been cylindrical, made with heavy-strength, flexible plastic wall materials, incorporated natural sediment for the bottom and located in open water habitats at depths of 2 to 10 m [9–11]. Enclosure volumes typically have ranged from 1 m^3 [12,13] to 1,300 m^3 [14–16]. Some enclosures have been built with rigid wall materials such as polyvinyl chloride [17] or stainless steel [18] and some have used the wall materials for the bottom, as in the CEPEX enclosures [19,20] and the studies conducted in large plastic bags [21–24].

A few efforts also have been made to enclose portions of the littoral zone [6,25,26], but, besides the littoral enclosure design presented here, only one attempt has been made to enclose an area that includes all of the habitat zones present in the littoral region (Mel Lungle, Mount Royal College, Calgary, Alberta, Canada, personal communication). These are the zone of emergent vegetation, the zone of rooted plants with floating leaves and the zone of submergent vegetation [27].

The enclosure design presented in this paper evolved from whole pond studies [28,29] and studies in larger enclosures [30]. The design was developed in response to a need by the United States Environmental Protection Agency (EPA), Office of Pesticide Programs, for improved hazard evaluation techniques for field testing of pesticides. Because of the need for new methodologies and the growing interest among the broader scientific community in developing reliable methods for conducting replicated experiments in natural aquatic ecosystems, the techniques for constructing littoral enclosures are described in some detail.

The potential utility of the design for assessing

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the impact of pesticide additions in replicated field experiments is evaluated based on some of the results of a study with the mosquito larvicide, Dursban® (chlorpyrifos), conducted during the summer of 1986. Because the coefficient of variation (C.V.) provides an estimate of experimental sensitivity or precision [31–33], the C.V.s associated with some of the chemical and biological measurements made during the 1986 littoral enclosure study are presented, as well as an overview of the major findings from this study.

MATERIALS AND METHODS

Construction

For our 1986 study, the littoral enclosures were rectangular (5 × 10 m), bordered with plastic walls on three sides and by 5 m of natural shoreline on the fourth side (Fig. 1a). They were constructed in three main steps. First, wooden walkways suspended on pairs of cedar posts fastened together with cross-supports were installed around the perimeter of the areas to be enclosed in the study

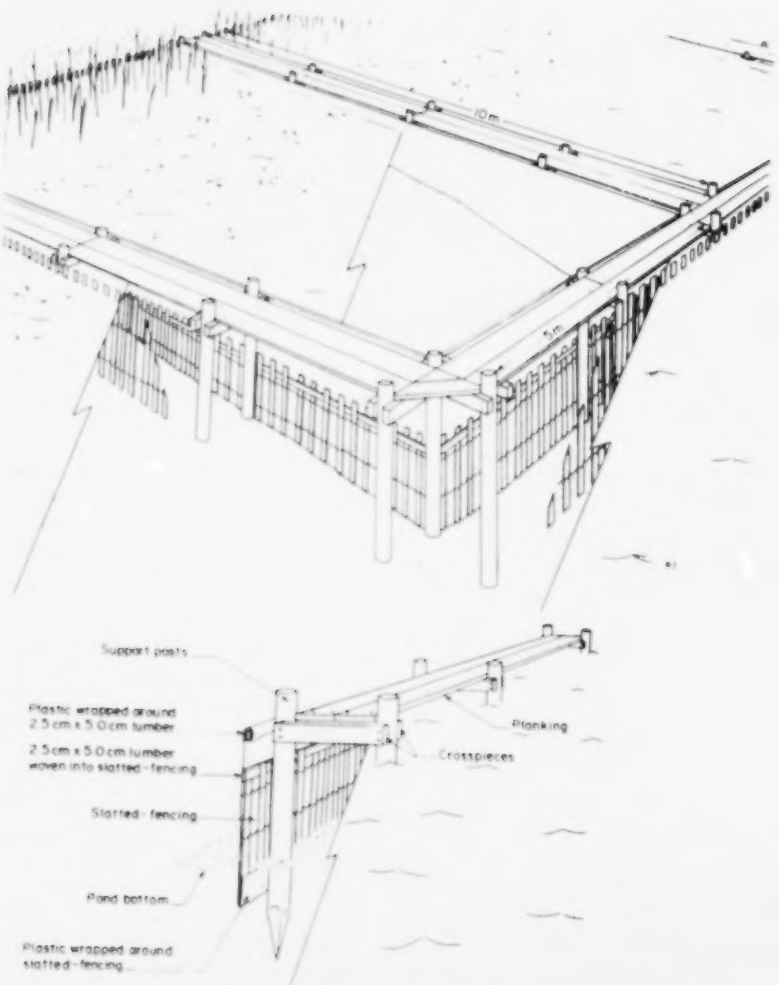

Fig. 1. Schematic diagram of a littoral enclosure in situ (a), with a cutaway view of an enclosure wall (a and b).

pond. The majority of this work was completed from a boat to assure that the natural sediments remained virtually undisturbed. Once completed, the walkways provided a stable platform for accessing the areas to be enclosed during further construction, and later for sample placement, sample retrieval and other research needs.

Next, walls of plastic and slatted fencing (snowfence) were assembled. The enclosure walls were constructed from an 0.20-μm, woven-filament, polyolefin plastic (#888 H UV Clear Scrimweve®, Sto-Cote Products, Inc., Richmond, Illinois), which is resistant to ultraviolet radiation, and untreated, wooden, slatted fencing (1.20 × 15.20 m). The width of the plastic was at least 1 m greater than the maximum water depth in each area to be enclosed. To construct an enclosure wall, rolls of slatted fencing were fastened together and trimmed to length and depth dimensions appropriate for the perimeter of the enclosure area. Wall length was adequate to allow for fluctuating water levels (depths) at the shoreline. The plastic was wrapped around and stapled to the slatted fencing along one edge (the edge that would be imbedded in the sediment during installation) to complete the wall.

Finally, for installation, the enclosure walls were positioned around the perimeter of the enclosure on the walkways, placed into the water and firmly pushed into the sediment at the base of the walkway support posts and all points in between the posts. Prior to stapling the slatted fencing to the walkway support posts, a piece of 2.5 × 5 cm lumber was woven between several of the slats at the top of the fencing, pushed firmly towards the pond bottom and nailed into two adjacent support posts (Fig. 1b). In soft sediments, such as those in our study pond, this assured a good seal between the enclosure wall and the pond bottom around the entire perimeter of the enclosed area.

Finishing the installation involved pulling the loose (top) edge of plastic taut, rolling it around 2.5 × 5 cm pieces of lumber to just below walkway level and securing the 2.5 × 5 cm lumber to support posts (Fig. 1b). The top edge of the enclosure walls could be unrolled and refastened to compensate for fluctuations in water level. By installing the walls along the inside perimeter of the walkways, adjacent enclosures were separated by 1 m of open pond water. This provided a water bath effect for the enclosures and prevented contamination between enclosures in case of leakage.

After installing the walls, an additional slatted fencing barrier was added around the entire outside perimeter of all the enclosures. This acted as a barrier to floating debris and was also intended to prevent access to the enclosures by wildlife. Finally, underwater inspection of the wall-sediment boundary was performed to assure that each enclosure was properly sealed.

Chlorpyrifos study

During 1986/87, a 12-enclosure design was used to study the effects of a single application of chlorpyrifos on the biota and physical and chemical environments within a mesotrophic, 2-ha pond near Duluth, Minnesota. Chlorpyrifos was applied at nominal concentrations of 0.0, 0.5, 5.0 or 20.0 μg/L. In this study, two enclosures were utilized for each of the control and low (0.5 μg/L) treatment groups and four enclosures were used for each of the medium (5.0 μg/L) and high (20.0 μg/L) treatment groups. The field experiments included studies on pesticide fate, degradation and dissipation and biological studies on the effects of pesticides on community structure and function (Table 1). C.V.s for the biological data were calculated from control enclosure samples collected during June, July and August 1986. The C.V.s for the chemistry measurements are based on data from all enclosures during this same time period. An overall mean C.V. averaged across all sample dates and replicates for each response variable and a range of C.V.s (minimum and maximum values observed on any given sample date) for each of these response variables were tabulated.

RESULTS AND DISCUSSION

Cost of the system

Constructing 12 littoral enclosures during 1986 cost less than $3,000 ($250 per enclosure) and required approximately 450 staff-hours to complete with a five-person staff for most tasks. Many of the components of this system are reusable. Wall plastic is the only major material item that must be replaced because of contamination from the experimental treatment and the biological community that develops on the enclosure walls over time. Reusability of most materials and structures reduces setup time and costs of future studies.

Structural integrity, flexibility and utility

The materials used to build the littoral enclosures and support structures are readily available throughout most of the United States, which should allow the design to be adapted to the littoral zone of most soft-bottomed ponds and lakes

Table 1. Chemical and biological studies conducted in littoral enclosures during the 1986 chlorpyrifos study[a]

	Measurement	Method
Water chemistry	Chlorpyrifos concentration	Liquid–liquid extraction, gas–liquid chromatographic quantitation
	NO_3, PO_4	Ion chromatography
	Dissolved oxygen	Potentiometric
	Dissolved organic carbon	Oxidative combustion
	Color	Platinum–cobalt comparitor
	Turbidity	Turbidimeter
	Conductivity	Potentiometric
	pH	Potentiometric
	Alkalinity	Potentiometric titration
Sediment chemistry	Chlorpyrifos concentration	Steam distillation extraction, gas–liquid chromatographic quantitation
	Organic carbon	Loss on ignition
	Grain size	Wet sieving
Bacteria	Total counts	Direct plate count
Phytoplankton	Chlorophyll *a*	Grab samples/fluorometric method
	Dry weight	
	Species composition and abundance	
Periphyton	Chlorophyll *a*	Substrate colonization/fluorometric method
	Dry weight	
	Species composition and abundance	
Macrophyton	Biomass	Quadrat counts/grab samples
	Species composition and abundance	
Zooplankton	Survival	In situ bioassay chambers
	Species composition and abundance	
Macroinvertebrates	Survival	In situ bioassay chambers
	Species composition and abundance	Artificial substrates
Fish	Survival	In situ bioassay cages
	Diet	Digestive tract content analysis
	Growth	Netted and trapped free-ranging fish
	Species composition and abundance	Traps
Litter decomposition	Weight loss	Litter bags
	Percent carbon remaining	Litter bags/elemental analysis
	Nitrogen content	
	Phosporous content	Litter bags/spectrophotometric method
System metabolism	Diel range of pH and dissolved oxygen	Continuous monitoring with in situ probes

[a] Adapted from Ref. [34].

without significant modification. The economy and durability of the building materials exceeded expectations and the strength and durability of the plastic and walkway support structures was excellent. Underwater inspection of the enclosure walls that were overwintered in situ revealed that the 1 m of ice and snow they were subjected to had not disturbed the wall–sediment seal, forced the walls away from the walkway support structures or punctured the plastic. Changes in water level did not affect the integrity of the system either. There was a 15-cm drop in the water level by mid-July 1986 due to low precipitation, but by mid-September the water level had returned to and eventually exceeded initial conditions (May 1986) by several cm. The enclosures also withstood a number of severe summer storms that included wind speeds of up to 100 km/h without a problem, and after 14 months in the pond the wall plastic did not appear to have deteriorated. This long-lasting structural integrity creates the potential for more long-term approaches to ecosystem studies within enclosures.

The slatted fencing makes an ideal support structure for the enclosure walls because of its vertical flexibility and light weight, which in turn makes transportation and installation relatively easy. The vertical flexibility allows the wall to conform to irregularities in the bottom contour that would not be possible with a rigid-bottomed wall. However, it is rigid enough to be imbedded in soft sediments and provide a good seal even where there is a heavy mat of vegetation. In sites with harder

bottom substrates, a sealing technique similar to the one described by Solomon et al. [7] might be more effective.

The basic littoral enclosure design described here has been used since spring 1985 to study the effects of pesticides on two different pond ecosystems [30,34]. These two pond study sites had very different shoreline topographies, depth profiles, biotic communities and watershed characteristics. The design was adapted successfully to enclosure sizes ranging from 50 to 150 m^2 in surface area and up to 1.5 m in depth. We feel that it could be extended into pelagic habitats if that component of the ecosystem were present and of interest, although this would require some modification of the wall structure to accommodate greater water depths. Adaptation to less remote, more controlled and uniform environments, such as hatchery ponds or other excavated ponds, should be relatively easy and may be the best application of this technique for performing replicated ecosystem experiments in lentic systems. The technique seems most appropriate for studies where the responses of communities that inhabit the littoral zone are of particular interest. For several reasons, it seems especially well suited for pond studies on the effects of pesticides. Littoral habitat often plays a key role in regulating whole-system metabolism [35], ponds tend to be dominated by littoral habitat where this enclosure system is designed to be used most efficiently and many pesticides' prime mode of entry into pond ecosystems is through runoff, aerial drift and/or direct application at the shoreline/littoral boundary.

1986 chlorpyrifos study

The actual effects that were detected during the chlorpyrifos study in littoral enclosures are summarized in Table 2. This semiquantitative format allows us to summarize a large amount of data associated with the many components investigated for this study. Several papers are currently in preparation that address the specific results for each of these components at length.

The effects that are noted were, in most instances, differences from control that were found to be statistically significant (analysis of variance (ANOVA) $p \leq 0.05$, Tukey's HSD) at some time during the first month after application. The phytoplankton and periphyton effects were production and biomass increases that were only statistically detectable within the first 8 d following treatment. These changes were probably an indirect response to the rapid reduction in invertebrate grazers following treatment. Many of the affected populations did not appear to have recovered completely at the end of the first season of study. Acute effects on juvenile bluegill sunfish survival were evident in the medium- and high-treatment groups, and a graded change in diet and growth was observed for endemic larval fathead minnows that was weakest in the low treatment enclosures and strongest in the high. Bacterial densities and decomposition rates had relatively low variability associated with their

Table 2. Summary of biological differences between control and chlorpyrifos-treated enclosures during the 1986 littoral enclosure study

	Nominal chlorpyrifos concentration, μg/L		
Measurement	0.50	5.00	20.00
Bacteria counts	0[a]	0	0
Phytoplankton			
Chlorophyll *a*	*	*	*
Species composition and abundance	0	0	0
Periphyton			
Chlorophyll *a*	0	0	0
Dry weight	*	*	*
Species composition and abundance	0	0	0
Macrophyton			
Biomass	0	0	0
Species composition and abundance	0	0	0
Zooplankton			
Cladoceran abundance	**	***	***
Copepod abundance	*	**	**
Rotifer abundance	**	**	**
Total abundance	**	**	**
Species richness	[b]	[b]	**
Macroinvertebrates			
Insect abundance	*	***	***
Amphipod abundance	**	***	***
Annelid abundance	0	0	0
Mollusc abundance	0	0	0
Total abundance	*	**	***
Species richness	0	[b]	**
Fish			
Survival	0	**	***
Diet	*	**	***
Growth	*	**	***
Litter decomposition	0	0	0
System metabolism	0	0	0

*,**,*** = gradation of statistically significant differences from control ($p \leq 0.05$), ranging from less than 25% (*), to between 25 and 75% (**), to greater than 75% (***).

[a]0 = no difference from control.

[b]Marked differences from control that were not statistically significant.

measurement and were apparently unaffected by the chlorpyrifos treatment, as was system metabolism. The fact that decomposition and system metabolism apparently were unaffected while many structural components of the system had undergone dramatic changes supports the idea that functional responses may be less sensitive indicators of change or damage in aquatic ecosystems [36].

It seems reasonable to use the coefficients of variation associated with the various chemical and biological measurements made during the 1986 chlorpyrifos study as an indication of the potential sensitivity of the littoral enclosure technique for detecting effects of pesticides on lentic systems relative to other experimental approaches. Several researchers have addressed the issue of acceptable levels of coefficients of variation for ecologically oriented microcosm experiments. Generally, C.V.s in the range of 20 to 30% were considered acceptable and normal [31,37], but it also has been pointed out that the acceptable level is highly dependent on the desired/required minimum detectable difference between control and treatment groups and the desired level of statistical power [32,33]. The smaller the magnitude of change between treatment and control that is considered ecologically significant and/or important to detect statistically and the higher the level of statistical power desired, the lower the acceptable level will be for the related C.V.s.

The C.V.s associated with measurements made for the 1986 chlorpyrifos study in littoral enclosures generally were between 10 and 40%, although some of the water chemistry, periphyton and fish values were higher (Table 3). These results are comparable to those observed in microcosm studies [31,33,37]. The relatively low C.V.s associated with most of the data from the chlorpyrifos study indicate that the littoral enclosure method can generate low variance data and suggest that it should be a sensitive tool to employ when attempting to detect the effects of pesticides or experimental treatments.

A more quantitative estimate of this sensitivity can be derived from the methods presented by Conquest [32]. Based on the approach she describes and the variability we observed during our 1986 study, a conservative estimate of the minimum percent change from control that one could expect to detect statistically with ANOVA on littoral enclosure data collected in a four treatment (control and three concentrations) by three replicate per treatment design would be between 20 and 40% for most variables. With a six treatment by two replicate design, the minimum detectable percentages would increase to between 40 and 75%, and with a three treatment by four replicate design they would decrease to between 15 and 30%. Because we were interested in detecting response differences between specified concentrations, a design that maximized replicates within treatment groups seemed most appropriate. However, if a gradation of responses across a wider range of concentrations was of more interest, the six treatment by two replicate design analyzed with a regression approach might be more appropriate. The specific objectives of the study and the percent change that is considered ecologically significant and important to detect need to be

Table 3. C.V.s associated with selected chemical and biological measurements made during the 1986 littoral enclosure study in June, July and August

	C.V.		
Measurement	Min Max	Overall mean	*n*
Chlorpyrifos chemistry			
Water	0.00–0.39	0.19	32
Sediment	0.02–1.12	0.37	9
Water chemistry			
Conductivity	0.02–0.59	0.24	28
pH	0.00–0.96	0.30	47
Alkalinity	0.00–0.35	0.17	47
Color	0.00–0.98	0.30	47
Turbidity	0.00–0.77	0.25	47
DOC	0.02–1.67	0.39	47
Bacterial counts	0.06–0.21	0.11	6
Litter carbon content	0.04–0.07	0.06	3
Phytoplankton			
Chlorophyll *a*	0.07–0.70	0.27	7
Periphyton			
Chlorophyll *a*	0.02–0.58	0.41	5
Dry weight	0.26–0.84	0.53	5
Zooplankton			
Cladocerans	0.03–0.37	0.10	19
Copepods	0.04–0.19	0.09	28
Rotifers	0.06–0.31	0.12	49
Total abundance	0.03–0.37	0.11	96
Macroinvertebrates			
Insects	0.02–0.41	0.14	30
Amphipods	0.10–0.33	0.24	3
Oligochaetes	0.09–0.23	0.14	4
Molluscs	0.19–0.32	0.24	3
Total abundance	0.02–0.41	0.16	40
Fish			
Length	0.11–0.17	0.14	16
Weight	0.37–0.61	0.50	16
Diet	0.16–1.63	0.86	48

Biological estimates derived from control data only, *n* = the number of C.V.s averaged for each overall mean.

factored into the decision of which experimental design to use.

Clearly, the sensitivity and effectiveness of the littoral enclosure technique as a tool for determining the effects of pesticides or other experimental treatments is dependent on many factors in addition to the C.V.s for the system components (e.g., number of experimental replicates; initial similarity of the physical, chemical and biological conditions; intensity and timing of the experimental treatment(s); natural variability; chance events). However, understanding the inherent variability of an experimental test system provides an opportunity to evaluate whether an experimental design will be adequate to detect treatment-related effects at the desired level of statistical significance and power. Ultimately, the problem of what constitutes a significant ecological change relative to a statistically significant change will have to be addressed. Continued analyses of the variability associated with a wide variety of both natural and experimental ecosystem components will provide a base of knowledge that could be used to help in making this determination. The ability to simultaneously monitor many response variables in replicate experimental units while maintaining a relatively natural environment and low cost makes the littoral enclosure technique seem like a very useful tool for these purposes. The U.S. EPA Office of Pesticides and Toxic Substances is currently revising the hazard evaluation guidelines for pesticide registrants to include a littoral enclosure approach as one of several field-testing options required "to support the registration of an end-use product that is likely to cause adverse long-term, cumulative, or life-cycle effects on fish or aquatic invertebrates" [38].

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